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Review

Cocaine pharmacology and current pharmacotherapies for its abuse

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Abstract—Cocaine abuse continues to be prevalent and effective therapies for cocaine craving and addiction remain elusive. In the last decade immunopharmacotherapy has been proposed as a promising means to alleviate this illness. By using the organism's natural immune response, an anti-cocaine vaccine promotes the production of cocaine-specific antibodies that sequester the drug before their passage into the brain, where it exerts its reinforcing and thus addictive effects. A series of studies demonstrating the cocaine-blocking properties of various immunogenic conjugates will be reviewed in the context of the neuropsychopharmacological profile of the drug.

Contents

1. Introduction

Cocaine abuse has produced a major epidemic health problem in North America in the 1980s, and has since become a serious medical and public health concern in the United States, with approximately 2.1 million people dependent on cocaine.[1]

The abuse of cocaine is maintained by the drug's effects on brain reward systems, and mediated at least in part by its dopaminergic action. The patterns and consequences of use are best understood by considering the pharmacokinetics (rapid absorption and delivery to the brain, relatively short half-life) and the pharmacodynamics (intense central and peripheral neural stimulation). Cocaine is used therapeutically as a topical and local anesthetic. Toxicity occurs primarily in cocaine abusers, but also occasionally after therapeutic dosing. Medical complications reflect primarily excessive central nervous system (CNS) stimulation and excessive vasoconstriction, the latter resulting in severe hypertension and/or organ ischemia with associated organ injury. Most deaths that result from medical complications of cocaine intoxication are sudden and occur before

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medical intervention is possible. Other complications of cocaine abuse with severe personal and social consequences include traumatic deaths and injuries, and reproductive disturbances, as well as transmission of infectious diseases, especially AIDS. Cocaine addiction represents a serious social and health problem, which has led the National Institute on Drug Abuse (NIDA) to urge the scientific research community for the development of an effective pharmacological treatment for this condition. However, pharmacotherapies of cocaine addiction that typically target the monoaminergic neurochemical substrates implicated in its reward properties, have as yet been unsuccessful, and often generate adverse side effects.[2]

Over the last decade, an alternate line of research has emerged aimed at treating cocaine addiction using immunological reagents and the immune system to peripherally block the effects of the drug. This approach addresses key components that constitute an effective treatment venue, such as a significant decrease of the rapidity of cocaine permeation into the brain while circumventing unwanted side effects. Early work demonstrated that antibodies specific for haptenic drugs were feasible and that they were useful in the attenuation of their effects.[3] In this study, a rhesus monkey trained to self-administer heroin and cocaine was immunized with a morphine hemisuccinyl-bovine serum albumin conjugate, that resulted in the gradual and selective extinction of heroin self-administration. Twenty years later, interest in this area was renewed with the first successful report describing effective blockade of the psychostimulant effects of cocaine by active immunization in rats.[4] The approach is based on the premise that cocaine by itself is not an immunogenic molecule, and as such cannot produce an immune response. However, if conjugated with an immunogenic carrier molecule, administration of the conjugate would induce the immune system to produce antibodies against cocaine. Therefore, anti-cocaine antibodies induced by a cocaine vaccine, by inhibiting the entry of cocaine into the brain, will inhibit the ability of cocaine to interact with all of its targets in the CNS. The specificity of the cocaine vaccine for the drug, rather than for its target, should also minimize interference with other therapies. Also, a therapeutic vaccine based on active immunization has the potential to provide long-lasting clinical efficacy for relapse prevention after administration and to have minimal problems with compliance in humans who are motivated to stop using cocaine.

The advent of new technologies for monoclonal antibody production and for creating highly specific human antibodies has brought these proteins into the realm of clinical evaluation. This alternative offers the immunotherapeutic application of passive transfer for the treatment of cocaine overdose. Other groups, as well as our own group, have explored alternate protein-based therapies involving the destruction of cocaine before it has a chance of reaching the brain such as murine monoclonal catalytic antibodies,[5–7] and cocaine metabolism enhancement with butyrylcholinesterase.[8]

This review focuses primarily on the development and testing of the immunotherapeutic reagents reported over the last decade. First, a general overview of cocaine neuropharmacology and pharmacotherapeutic research to date will be presented. Next, we offer a perspective on the challenges to be faced in the successful application of the immunotherapeutic technologies thus far developed. Finally, other protein-based, nonpharmacological cocaine blockers will be explored.

2. Cocaine pharmacology

Cocaine is obtained from coca leaves (containing between 0.6% and 1.8% alkaloidal cocaine) using a relatively simple method by which it is extracted from the leaves with an organic solvent (often kerosene), resulting in a coca paste containing about 80% cocaine. The alkaloids are passed through an acidic aqueous solution based on hydrochloric acid; the solution is neutralized and the cocaine is extracted by recrystalization. Cocaine hydrochloride (HCl) is the pharmaceutical form used as a local anesthetic, and abused by drug addicts. It is readily water-soluble and thus can be taken orally, intranasally, or by intravenous injection. Cocaine HCl is vulnerable to pyrolysis, resulting in a poor rewarding effect when smoked. However, upon transformation into cocaine freebase, (popularly termed 'crack' or 'rock' cocaine) by dissolving it in an alkaline solution, followed by precipitation, the hydrochloride salt transforms into a smokable, pyrolysis-resistant material.

Different routes of consumption yield somewhat different patterns and levels of plasma cocaine concentration. Extremely rapid absorption occurs with both intravenous injection and smoking. Hence, typical single doses taken by the routes yield rather high concentrations of circulating cocaine (500–1000 ng/mL),[9,10] although even higher values can be attained with multiple doses that mimic the pattern of a cocaine 'binge'.[11] With a pK_a of 8.7, orally consumed cocaine tends to be ionized in the digestive system, slowing the rate of absorption. Also, snorting results in slower absorption; consequently these routes yield lower cocaine levels in the range of 100–500 ng/mL.

The likelihood that a person will become addicted to cocaine depends to a large degree on the method, frequency and duration that a person used the cocaine. One study conducted by the Outpatient Recovery Centers found that cocaine smokers were twice as likely to fail to complete their treatment program compared to intranasal abusers. Other elements that determine addiction liability are: the psychological and physical changes brought about by drug use, the degree of change, the speed of onset of the change, the duration of change, and the post-drug effects.[12] Given the characteristics that define the probability of addiction, cocaine is most addictive when it is smoked. Cocaine can be smoked as coca paste, as freebase, or as crack. The popularity of crack compared to freebase is largely a product of marketing techniques, which make small

amounts of high-quality cocaine available at low prices and without having to undertake a dangerous chemical process to convert cocaine to a smokable form. In fact, smoking is not an efficient method of delivering cocaine to the body. A significant portion of the drug dose is lost when the cocaine is heated in preparation to be smoked. The effects of the drug come on very quickly; only 8–10 s pass before the user experiences the high. The peak concentration of the drug in the brain also occurs more rapidly when smoked, resulting in greater behavioral effects for a shorter period of time. Also contributing to the reinforcing and addiction potential of crack is the fact that the effects of the drug last only 8–10 min. After the high is over, the crack user feels anxious, depressed, and paranoid. Such a rapid shift between positive and negative effects of the drug make users crave another 'hit' of the drug to restore the euphoria they felt just moments before.[13]

Cocaine metabolizes primarily into ecgonine methyl ester (**2**) and benzoylecgonine (**3**, Fig. 1), which are renaly excreted. Both metabolites, account for 75–90% of cocaine metabolism.[9] At a smaller scale, ecgonine and the biologically active metabolite norcocaine are also formed. Upon breakdown of cocaine to ecgonine methyl ester, rapid catalysis by serum and liver cholinesterases occurs, while hydrolysis of the ethyl ester linkage to form benzoylecgonine may be nonenzymatic.[14] Finally, the demethylation of cocaine to form norcocaine (**4**) takes place through the hepatic mixed function oxidase system.

Turning to the pharmacokinetics, the half-life of cocaine is short, approximately 90 min, because it is rapidly metabolized by the liver, which leads during its first passage to the production of benzoylecgonine. Renal elimination of cocaine and benzoylecgonine is prompt and is greater if there is an acidic urine pH, whereas the metabolite is eliminated preferably at an alkaline pH. Metabolic detoxification of cocaine is promoted by the plasmatic and hepatic cholinesterases, whose fluctuations in activity (for instance, in the fetus, the newborn child, during pregnancy, old age, in congenital cholinesterase deficiency) can reflect unexpected variations in the intensity of the pharmacological and toxicological response to cocaine.

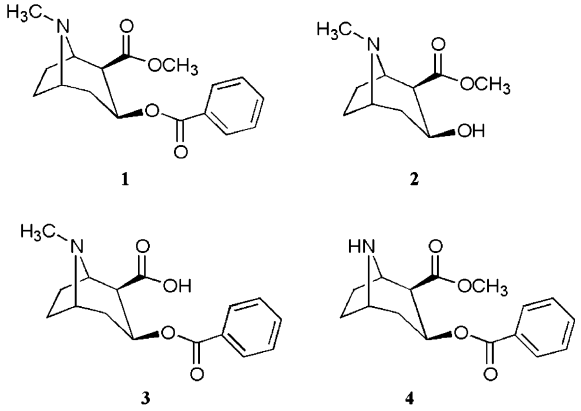

Figure 1. Cocaine (**1**) and its metabolites ecgonine methyl ester (**2**), benzoylecgonine (**3**), and norcocaine (**4**).

In studies using intravenous injections, variable estimates of half-life elimination of cocaine range from 16 to 90 min across different individuals.[14] Despite this variability, the rapid clearance of cocaine results in the subjective 'high' produced by a single intravenous or smoked dose of cocaine may last only about 30 min.[15] It has been hypothesized that this pharmacokinetic feature is a key component in the high addictive value of cocaine, and thus merits special consideration in the development of therapeutic strategies to treat its abuse and relapse potential.

3. Cocaine neuropharmacology

The two main health-hazards associated with cocaine consumption are the abuse of the drug and the toxicity/lethality as a result of overdose. The neuropharmacology of both of these conditions focuses on the actions of cocaine upon the discrete aspects of the CNS and disruptions of cerebral blood flow. The effects of cocaine on the CNS and on cerebral blood flow are complex and only partly understood. Cocaine alters synaptic transmission by interacting with the plasma membrane transporters for dopamine (DA), norepinephrine (NE), and serotonin (5HT), blocking cellular uptake, which in turn enhances monoaminergic synaptic activity by increasing transmitter availability within the synaptic cleft.[16–18] Actions at the DA transporter are believed to be most important for the reinforcing effects of cocaine; for example, mice with a null mutation in the DA transporter gene are much less sensitive than normal mice to the behavioral effects of the drug.[19]

The major subjective and behavioral effects of cocaine are summarized in Table 1. Low to moderate doses of cocaine in naive subjects or in users who have not yet progressed to heavy, chronic patterns of drug intake entail a behavioral and subjective aftermath of a positive, powerfully reinforcing nature such as euphoria and hypermotility. Many of these positive effects become

Table 1. Mild to moderate versus severe behavioral and subjective effects of cocaine in humans

Mild-moderate effects	Severe effects
Mood amplification; both euphoria and dysphoria	Irritability, hostility, anxiety, fear, withdrawal
Heightened energy	Extreme energy or exhaustion
Sleep disturbance, insomnia	Total insomnia
Motor excitement, restlessness	Compulsive motor stereotypies
Talkativeness, pressure of speech	Rambling, incoherent speech
Hyperactive ideation	Disjointed flight of ideas
Increased sexual interest (libido)	Decreased sexual interest
Anger, verbal aggression	Possible extreme violence
Mild to moderate anorexia	Total anorexia
Inflated self-esteem	Delusions of grandiosity

negative or aversive with escalation of cocaine dose and duration, and some of these effects are present in most high-dose users. When taken chronically, cocaine may lead to stereotypic (frantic and repetitive) motor responses.[10,20] Therefore, the magnitude of the reinforcing properties of cocaine intake are directly correlated to the dose of the drug and, as mentioned in the previous section, route of administration.

A wealth of research has demonstrated that cocaine exerts its reinforcing and thus addictive effects within the neurocircuitry of the mesocorticolimbic DA system.[21] The most widely used animals models used to demonstrate the neural correlates of cocaine's psychoactive and reinforcing effects are the locomotor activity assay and intravenous self-administration. There is substantial evidence linking cocaine-induced locomotor activation to blockade of DA reuptake. Kelly and Iversen first reported reductions in cocaine-induced locomotor activity, and many studies since have supported this hypothesis showing that locomotor activity can be elicited by direct microinjections of cocaine in the nucleus accumbens, a structure found within the mesocorticolimbic DA system.[22,23]

The neural mechanisms of cocaine reinforcement have been most extensively studied using the self-administration paradigm. Early studies showed that low doses of DA antagonists increased the rate of cocaine self-administration, whereas high doses of the same compounds led to a cessation of responding.[24,25] Moreover, cocaine self-administration is attenuated by 6-OHDA lesions of either the nucleus accumbens[26] or the ventral tegmental area (VTA), the site of the dopaminergic neurons that project to the accumbens.[27] Consistent with these findings, in vivo microdialysis experiments have shown that cocaine self-administration leads to increased extracellular DA concentrations in the nucleus accumbens.[28] Therefore, most of the behavioral effect of cocaine in animals have been attributed to activation of dopaminergic transmission, particularly in the nucleus accumbens.

Cocaine produces its toxic effects and a variety of other physiological and behavioral effects through its interactions with several distinct CNS receptor sites. Additional to the monoaminergic effects of cocaine, receptor-binding studies have shown that (−)-cocaine interacts with both sigma and muscarinic cholinergic receptors in the brain.[29] Other findings of interest are that cocaine does not appear to inhibit ligand binding to GABA, NMDA, phencyclidine, or benzodiazapine receptors among others, receptors related to neuronal systems typically associated with seizures, as well as to the efficacy of various anticonvulsant compounds.[29,30]

On the cerebral vascular system cocaine seems to act directly, its action being mediated mainly by the effects on noradrenergic neurotransmission. Cocaine causes vasoconstriction, decrease in cerebral blood flow,[31] and inflammation in the walls of the brain vessels (vasculitis).[32,33] Other chronic disorders have been associated with the use of cocaine. Among these, partial or generalized epileptic seizures,[34] believed to be a consequence of cocaine-induced hyperpyrexia, and the reduced cerebral blood flow observed after cocaine intake, or heart disorders caused by the drug, such as tachycardia and ventricular fibrillation.[35]

The above cited studies show that the neuropharmacological correlates of the addictive and toxic effects of cocaine occur mainly within the confines of the CNS. This knowledge has laid the grounds for an extensive body of pharmacological research aimed at developing a therapeutic means to alleviate the physical and mental afflictions caused by cocaine abuse.

4. Current pharmacotherapies

The development of pharmacotherapy for cocaine addiction is based on previous strategies designed to alleviate other chemical dependencies such as alcoholism and opiate addiction, focusing on the neurobiological and the behavioral bases of addiction.[36] To date, no pharmacotherapy has been approved by the U.S. Food and Drug Administration for cocaine dependence, but two major classes of medications have been investigated: (1) dopaminergic agents and (2) antidepressants. Studies have been relatively brief for both types of agents and have focused on abstinence initiation rather than on relapse prevention. In addition to dopaminergic agents and antidepressants, other compounds such as calcium channel blockers, have been examined as potential treatments of cocaine dependence.

4.1. Dopaminergic agents

Based on the theory that chronic cocaine use reduces the efficiency of central DA neurotransmission (vide supra),

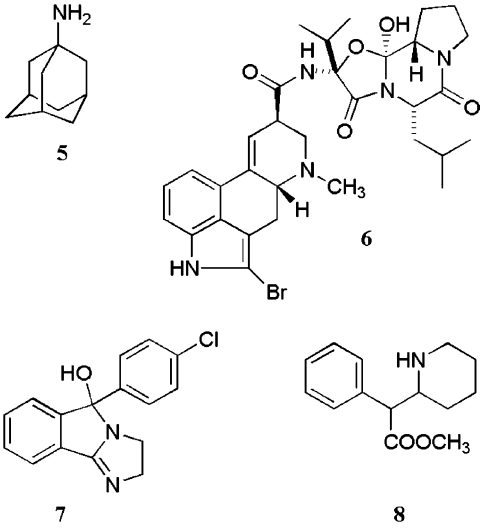

Figure 2. Dopaminergic agents: amantadine (**5**), bromocriptine (**6**), mazindol (**7**), and methylphenidate (**8**).

several dopaminergic compounds, including amantadine (**5**), bromocriptine (**6**), mazindol (**7**), and methylphenidate (**8**) (Fig. 2), have been examined as treatments for cocaine abuse. Investigators hoped that these dopaminergic agents, which have a fast onset of action, would correct the DA dysregulation and alleviate the withdrawal symptoms that often follow cessation of stimulant use.

A large array of cocaine analogues and other dopamine uptake inhibitors (see Table 2) including analogues of WIN-35,065 (**9**), GBR-12909 (**10**), nomifensine (**11**), and benztropine (**12**) (Fig. 3) have been developed in the last 15 years.[37–46] The largest class of compound studies is the class of 3-phenyltropane analogues, of which many hundreds have been made and tested. The analogues RTI-112 (**13**) and PTT (**14**) (Fig. 4) are in preclinical evaluation. Like cocaine, RTI-112 and PTT both have good affinity for all three monoamine transporters, but in contrast to cocaine they enter the brain slowly and are long-lasting. A number of other 3-phenyltropanes are potent and selective for the dopamine transporter relative to inhibition of serotonin and norepinephrine

Table 2. Studied pharmacotherapeutic compounds for cocaine abuse

Compound	CNS targets
RTI-113, RTI-177, GBR-12909	DA uptake inhibitors (selective for DAT)
WIN-35,065, RTI-112, PTT, mazindol, methyl phenidate, nomifensine, benztropine	DA uptake inhibitors (not selective)
Apomorphine, bromocriptine, SKF38393, SKF82958, 7-OHDPAT, quinpirole	DA receptor agonists and partial agonists
Desipramine	Antagonists of cocaine binding that spare DA uptake
Fluoxetine, alaproclate	5HT uptake inhibitors
Quipazine	5HT receptor agonist
Ketanserin, ritanserin, MDL-72222, ondansetron	5HT antagonist
Isradipine	Calcium channel blockers

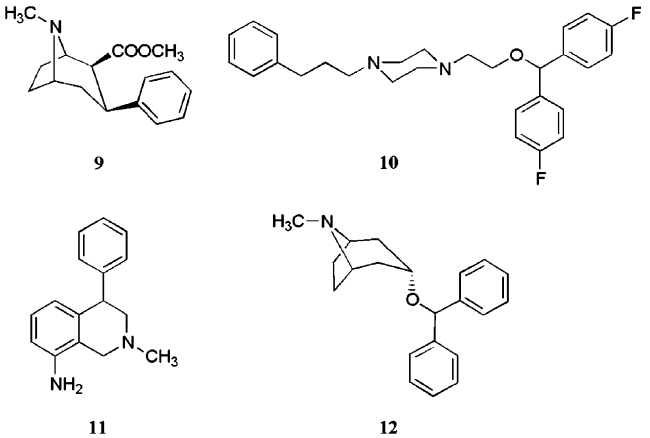

Figure 3. Dopaminergic agents: WIN-35,064 (**9**), GBR-12909 (**10**), nomifensine (**11**), and benztropine (**12**).

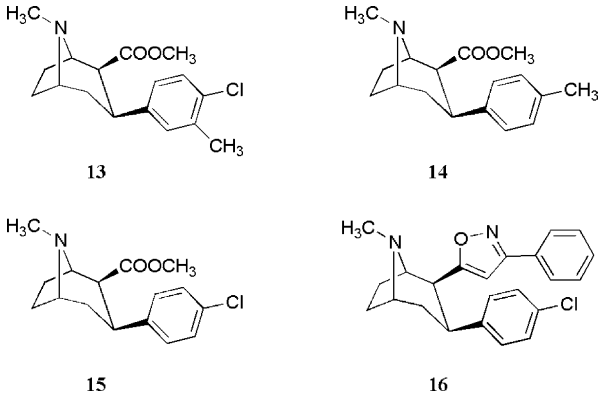

Figure 4. Dopaminergic agents: RTI-112 (**13**), PTT (**14**), RTI-113 (**15**), and RTI-177 (**16**).

transporters, are long-lasting, and also enter the brain more slowly than cocaine,[47] for example, RTI-113 (**15**) and RTI-177 (**16**) (Fig. 4).

Compounds of the GBR series are also known to be potent and selective for the dopamine transporter and have been considered as viable substitute agonist medications.[37,48] Preclinical studies of these compounds in nonhuman primates, however, have also indicated the potential for abuse in humans,[49,50] which may be a problem with all substitute agonists.

The effect of inhibiting neurotransmitter uptake is to stimulate neurotransmitter receptors; thus the use of direct receptor agonists as substitute agonist medications also has been suggested. Data on dopamine receptor agonists have been extensively reviewed. Animal studies indicate that dopamine receptor agonists such as apomorphine (**17**) (Fig. 5) and bromocriptine (**6**) maintain self-administration in rodents.[51,52]

A number of studies have found amantadine (**5**) to be effective in attenuating cocaine craving and use. For instance, in cocaine-abusing methadone-maintained patients, Handelsman et al. reported decreased urine

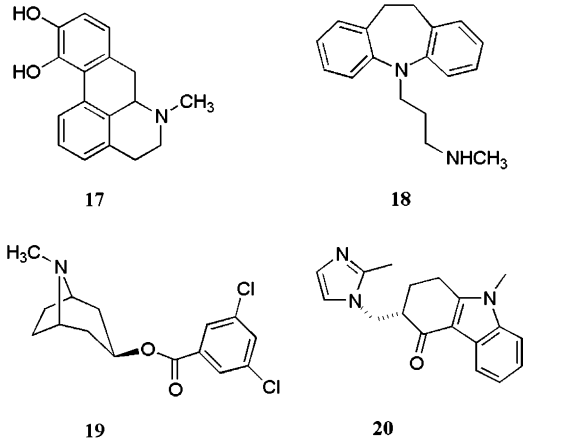

Figure 5. Dopaminergic agents and antidepressants: apomorphine (**17**), desipramine (**18**), MDL-72222 (**19**), and ondansetron (**20**).

cocaine metabolite levels and self-reported cocaine use following amantadine treatment,[53] and Alterman et al. found that patients who were given amantadine had significantly greater rates of cocaine abstinence than did placebo-treated subjects.[54] A double-blind comparison of amantadine and bromocriptine for the treatment of acute cocaine withdrawal evidenced that amantadine was superior to bromocriptine.[55] However, a placebo-controlled study comparing amantadine and placebo observed no differences between the treatment groups.[56] Amantadine has also been compared with desipramine (**18**) (Fig. 5) and a placebo. In methadone-maintained patients, no differences were found among the three groups in treatment retention, cocaine craving, and cocaine-free urine samples, but attainment of two weeks of cocaine abstinence was significantly greater in the amantadine treatment group relative to the desipramine or placebo groups.[57] Thus, these studies suggest that amantadine would be only marginally effective in reducing cocaine withdrawal symptoms as initial attainment of cocaine abstinence.

The DA D2 agonist bromocriptine (**6**) also has been examined in the treatment of cocaine dependence. In a double-blind, placebo-controlled study with cocaine-dependent individuals, Giannini and Billett found that compared with placebo, bomocriptine significantly reduced cocaine craving,[58] while another report noted that bromocriptine blocked the euphorigenic effects of cocaine in several addicted patients.[55] Despite these positive results, bromocriptine has been reported to produce side effects such as headache, nausea, hypotension, and psychosis,[59,60] which makes this agent a poor treatment choice.

Mazindol (**7**), a catecholamine reuptake inhibitor, was initially associated with reduced cocaine use,[61] but more recent studies failed to show significant therapeutic effects. For instance, in a one-week crossover study, mazindol did not reduce cocaine use or craving,[62] and in double-blind studies, no differences were observed between mazindol and placebo treatment groups in self-report ratings, rates of relapse, or cocaine use.[63,64]

Methylphenidate (**8**), a stimulant, has been examined for treatment of cocaine dependence because of its low abuse liability relative to cocaine or amphetamine, rapid onset of action relative to antidepressants, and long duration of action relative to cocaine.[57] Methylphenidate has been reported to produce beneficial effects in cocaine-dependant individuals with attention-deficit/hyperactivity disorder (ADHD).[65] However, others have found that methylphenidate results in a mild sense of stimulation that evokes an increased desire for cocaine in persons without ADHD.[66] Furthermore, a recent double-blind, placebo-controlled study found no difference in cocaine use between methylphenidate and placebo treatment groups.[67]

Overall, these studies indicate that the rewarding effect and withdrawal symptoms induced by cocaine may be modestly attenuated by fast-acting dopaminergic agents. However, the significant side effects resulting from alterations to such a preponderant neurochemical system, decrease the medicinal value of these compounds.

4.2. Antidepressants

The second class of medications used to treat cocaine dependence, antidepressants, are thought to downregulate synaptic catecholamine receptors, and this action is opposite to the presynaptic upregulation caused by chronic stimulant use.[68] Although antidepressants have a relatively benign side-effect profile, good patient compliance rates, and lack of abuse liability, they have a delayed onset of action ranging from 10 to 20 days.[62] Therefore, the physician may consider beginning antidepressant treatment during early withdrawal and continuing for weeks or longer as clinically indicated.

The tricyclic antidepressant desipramine (**18**) has been studied most extensively as a treatment of cocaine dependence. Early studies of desipramine to treat cocaine dependence showed positive results,[55,68] but placebo-controlled trials have not produced impressive findings. A meta-analysis of placebo-controlled studies by Levin and Lehman showed that although desipramine did not improve retention in treatment, it did produce greater cocaine abstinence relative to placebo.[69] However, treatment with desipramine has induced 'early tricyclic jitteriness syndrome' and cocaine craving, as well as relapse to cocaine use in some patients.[70] Therefore, desipramine as pharmacotherapy would hold serious clinical caveats.

Additional studies have focused on the involvement of the 5HT3 receptor subtype in the neuropharmacology of cocaine, but the results obtained are somewhat inconsistent. Several 5HT3-selective antagonists, including MDL-72222 (**19**) and ondansetron (**20**) (Fig. 5) were reported to attenuate cocaine-induced locomotor activity in rodents.[71] However, ondansetron failed to block the reinforcing or discriminative-stimulus effects of cocaine in rodents.[72,73]

Several other antidepressants, including fluoxetine (**21**), sertraline (**22**), and trazodone (**23**) (Fig. 6), that work predominantly through serotonergic mechanisms also have been used as pharmacotherapy for cocaine dependence. Although some reports indicated that

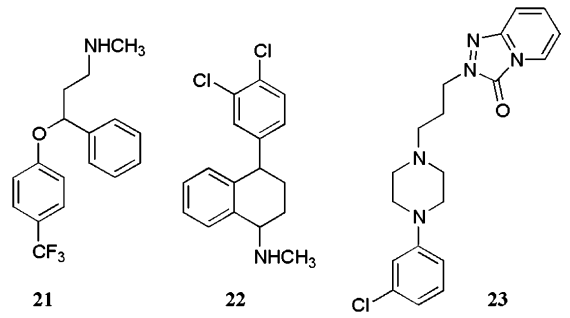

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Figure 6. Antidepressants: fluoxetine (**21**), sertraline (**22**), and trazodone (**23**).

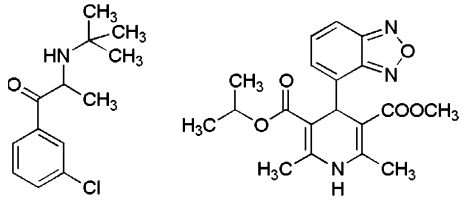

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Figure 7. The antidepressant bupronion (**24**) and the calcium channel blocker isradipine (**25**).

treatment with fluoxetine reduced cocaine craving and use in cocaine-abusing heroin addicts,[74,75] other investigators have not found fluoxetine to be effective in attenuating cocaine use and withdrawal symptoms.[57,76] Bupropion (**24**) (Fig. 7), a 'second-generation' antidepressant, has been studied as pharmacotherapy for cocaine dependence. In an open pilot study, treatment with bupropion reduced cocaine use[63] and attenuated several cocaine-induced onsite subjective ratings during laboratory cocaine administration.[77] However, another study failed to show that bupropion produced greater efficacy than placebo for reducing cocaine's effects.[78]

4.3. Calcium channel blockers

Various studies suggest that L-type calcium channel blockers potentially reduce the rewarding effects of cocaine. One such compound, the L-type calcium channel blocker isradipine (**25**) (Fig. 7), attenuated the cocaine-induced dopamine release in the striatum of rats.[79] Another report described isradipine-induced attenuation of condition place preference and the discriminative-stimulus properties of cocaine.[80] Also, pretreatment with isradipine resulted in a dose-dependent decrease in intravenous cocaine self-administration.[81,82] Because of the antihypertensive quality of calcium channel blockers, the potential increase in cardiac output in patients with normal ventricular function could complicate their use as pharmacotherapies for cocaine abuse.

Notwithstanding the impressive amount of research effort in this area, a large number of studies using dopaminergic drugs have failed to yield encouraging results. To date, no pharmacotherapeutic agent of this type used on an experimental basis has been shown effectiveness that would merit medical implementation.

5. Induction of the immune response

The importance of natural immunity has been widely recognized and defined by the generation or presence of protective, therapeutic antibodies to infectious or foreign agents.[83–85] Antibody responses make up the humoral response, and immune protection from disease can be passively transferred through serum containing antibodies. Antibodies represent a dominant class of serum proteins; reaching 10 mg/mL in the serum following immunization or during the course of severe chronic infections, or as a result of proliferative diseases of antibody producing cells. The major classes of immunoglobulins (Ig) include IgG, IgA, IgM, IgD, and IgE.[86] IgG represents the protective, high affinity antibodies circulating in the blood (with a half-life of about three weeks) with neutralizing activity for infectious agents, bacteria, viruses or soluble toxins, and target antigens in general. IgG antibodies are large molecules, having a molecular weight of approximately 150 kDa, are composed of two different kinds of polypeptide chains[86] (Fig. 8). One, of approximately 50 kDa, is termed the heavy or H chain, and the other, of 25 kDa, is termed the light of L chain. The two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. In any given immunoglobulin molecule, the two heavy chains and the two light chains are identical, giving an antibody molecule with two identical antigen-binding sites, and thus the ability to bind simultaneously to two identical structures. An antibody is composed of multiple Ig domains. A heavy chain contains four Ig domains, while the light chain has only two. The N terminal Ig domain of a heavy chain and light chain aligns in the Ig molecule and forms the antigen-binding pocket. One IgG molecule has two antigen-binding sites, and this portion of the protein can be separated from the constant region by enzymatic digestion, which cleaves the molecule to the antigen binding $F(ab)_2$ and the constant Fc fragments.[86]

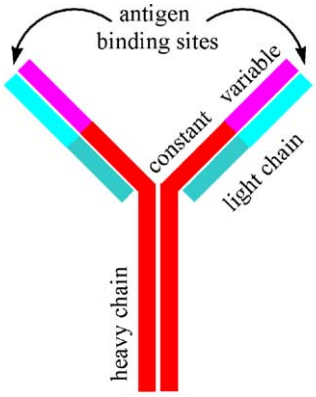

Figure 8. Schematic representation of IgG antibodies.

The peptide sequence of the N terminal Ig domain is a variable property achieved by recombination and somatic mutation mechanisms. The two variable segments that together make the antigen-binding pocket contain peptide sequences of key importance in the complementarity determining regions (CDRs) responsible for interaction with the antigen.[87] CDRs represent the most variable peptide sequences in the Ig molecule and are coded by variation, diverse and joining genomic segments. The total possible combinations of various segments and somatic mutation are responsible for the diversity of antigen-binding sites that exceeds 106 possible permutations. Invariant parts of Ig chains are class IgD, IgM, IgE, IgA, and IgG subclasses. Ig classes differ in their effector activities, which are associated with the

distal, Fc portion of the molecules. Specific Ig binding receptor mediated activation or inhibition of macrophages, granulocytes and mast cells, as well as intestinal, endothelial and placental transfer of Ig molecules are dependent on various forms of Ig class specific Fc receptors.

IgM is the predominant immunoglobulin of the primary immune response after challenge by an unknown antigen for the very first time.[86] The pentameric backbone, with ten antigen-binding sites, is very effective in agglutination of antigenic particles. Moreover, IgM is the most potent isotype in activating the complement cascade, a system of serum proteins supporting many immunological functions. The monomeric IgM variant is anchored on the surface of B cells and serves as the specific B cell receptor (BCR). Together with the surface IgM molecule, IgD is coexpressed on the outer membrane of B cells.

IgG is the predominant antibody of the secondary immune response.[88] It is produced rapidly in high concentrations after meeting an already known antigen. During the first contact, B cells specific for the appropriate antigen expand and produce IgM antibodies. Moreover, a few of these specific B cells do not proliferate but rather develop into memory cells. The latter are long living (up to 10 years) cells with the same specific BCR. These primed cells are ready to react when the appropriate antigen is presented and they start to divide into further memory cells and many effector B cells, producing IgG antibodies without having to perform all of those recombination steps. This memory effect of lymphocytes (the same is true for T lymphocytes) is the basis of successful vaccination.[86]

6. Anti-cocaine haptens developed and preclinical studies

In the last 10 years, a new therapeutic strategy against cocaine abuse has been explored. This approach entails the synthesis of a therapeutic cocaine vaccine that induces the production of anti-cocaine antibodies. This immunogenic response is hypothesized to bind peripherally circulating cocaine, forming a large molecular complex, impenetrable through the blood–brain barrier, thus impeding its passage into the CNS, where the drug exerts its addictive effects. As a result, the reinforcing value of cocaine is expected to decrease, manifested in a significant reduction of relapse behavior (Fig. 9).

Cocaine itself cannot induce an immune response, but covalent linkage with a carrier protein makes it a haptenic molecule affording it immunogenic properties. The carrier protein serves a dual function: it stimulates T-cell mediated antibody production while providing a scaffolding, rendering cocaine multivalent and able to cross-link immunoglobulin on the surface of B cells. Similar technology is currently used for various conjugate vaccines presently available on the market, including the series of *Haemophilus influenzae* type b vaccines, which couple polysaccharide antigens to protein carriers.[89]

Although several carrier proteins can be used in a conjugate vaccine, cocaine vaccines reported in the last decade have been synthesized by conjugating a conjugate analog or cocaine itself to KLH (keyhole limpet hemocyanin)[4,90] or bovine serum albumin (BSA).[91] Using this methodology, Basagra et al. achieved anti-cocaine antibody induction in rats, reaching serum levels ranging from 0.004 to 0.019 mg/mL. In these rats, the analgesic effects of 25 mg/kg cocaine were reduced. Furthermore, levels of circulating antibody negatively correlated with the reaction time on the hotplate analgesia test. However, none of the animals showed complete resistance to this single moderate dose of cocaine, thus it was suggested that the hapten, immunization dose, and regimen were not optimal. This study was followed by the first successful report of a true blockade of the psychostimulant effects of cocaine by active immunization by Carrera et al.[4] In this study, the cocaine-KLH conjugate, GNC-KLH (**26**) (Fig. 10), was shown to suppress locomotor activity and stereotyped behavior induced by systemic cocaine (15 mg/kg) but not amphetamine. Furthermore, vaccination with GNC-KLH resulted in significant decrease of cocaine levels in brain tissue of up to 80%. In a later report,[92] it was demonstrated that antibody titers rose to over 25,000 in

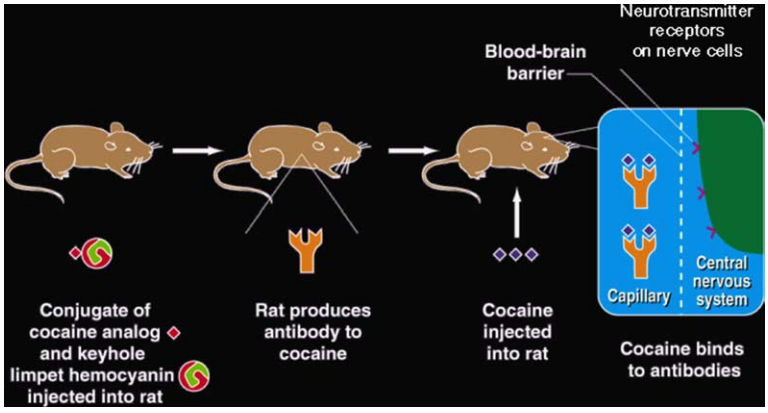

Figure 9. Schematic representation of anti-cocaine vaccination strategies.

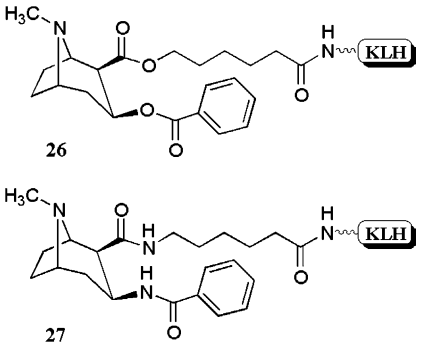

Figure 10. Cocaine-KLH immunoconjugates GNC-KLH (**26**) and GND-KLH (**27**).

rats given three injections of 250 μg GNC-KLH over a five-week immunization period. This level of antibody was sufficient to block reinstatement of responding induced by a single noncontingent priming infusion of cocaine, it also proved moderately surmountable when a threefold increase in cocaine dose was available. Upon ad libitum exposure to cocaine elicited responding of approximately 200% above baseline. Of note, this dramatic rise in responding was entirely blocked by iv administration of the monoclonal anti-cocaine antibody (mAb) GNC-92H2 at the 15 and 30 mg/kg dose. Importantly, active immunization with GNC-KLH resulted in a rightward shift of the ascending limb of the U-shaped dose–response curve, suggesting that this treatment possesses true therapeutic potential for the abatement of cocaine abuse.

Subsequent work by this same group developed a second-generation cocaine-KLH conjugate termed GND-KLH (**27**). Immunization (3×250 μg over five weeks) resulted in a dramatic suppressing of the psychomotor stimulant effects of 15 mg/kg cocaine.[93] As this effect was sustained for up to 12 days after immunization, GND-KLH was an important improvement over GNC-KLH by offering longer-term protection against cocaine. As the antagonistic effects of the immunization protocol were not absolute, it was concluded that the anti-cocaine antibody titers generated by this treatment are still surmountable by increasing doses of the drug. However, to date these data reflect the most significant blockade of cocaine by any immunotherapeutic means. Other groups implemented similar cocaine-KLH haptens in the efforts to blunt the analgesic effects of the drug, meeting with limited success.[94,95] In these studies, cocaine was bound to KLH for immunization with a photoactivatable cross-linker (N-hydroxysuccinimide-4-azidobenzoate). This conjugation method likely resulted in several different orientations of the exposed cocaine molecule, possibly resulting in a broad spectrum of anti-cocaine antibodies. Immunization with this cocaine-KLH complex resulted in a marginally attenuating effect of the analgesic and reinforcing properties of cocaine in laboratory rodents.

A later study by Fox et al. tested the effect of an anti-cocaine vaccine termed IP-1010 (3×10 μg over six weeks) in rats trained to self-administer 1 mg/kg cocaine, at various doses of the drug.[91] Serum antibody levels ranged from 0.008 to 0.709 mg/mL two weeks after the last vaccine injection. Only rats having serum antibody levels greater than 0.05 mg/mL displayed attenuated drug-seeking behavior and drug infusions across the range of doses examined. The ascending limb of the inverted U-shaped dose–response curve was shifted to the right for both drug-seeking behavior and number of drug infusions earned, suggesting that immunization with the cocaine vaccine antagonized the reinforcing effects of cocaine in this group of animals. However, valid concerns were raised with regard to the schedule of reinforcement and behavioral paradigm implemented for this,[96] which makes the interpretation of these data controversial.

There is clearly a need for new treatment options for cocaine addiction because the relapse rate among drug abusers seeking treatment is quite high. The described preclinical studies offer an optimistic scenario for the successful application of this therapeutic approach. It will remain to be seen if clinical trials demonstrate the safety and efficacy of treating cocaine addiction with immunopharmacotherapy.[57]

7. Other nonpharmacological cocaine-blocking agents

In addition to both active immunization and passive administration of monoclonal antibodies used to develop potential protein-based therapies for cocaine addiction, other approaches involving peripheral modifications to the cocaine molecule have been explored. One such approach entails the destruction of cocaine before it has a chance of reaching the brain. An alternative form of passive immunization is the passive transfer of monoclonal catalytic antibodies that bind cocaine and subsequently hydrolyze the alkaloid into its inactive products ecognine methyl ester and benzoic acid (Fig. 11). The unique feature of this preparation is that after it hydrolyzes cocaine and releases its metabolites, the antibody becomes free for further binding. To obtain antibodies able to catalyze this reaction, a transition-state (TS) analogue approach for hapten design has been reported,[5–7] and these immunogens would elicit the first enzymes able to degrade cocaine in vitro. In this model, the benzoyl ester at C-3 of the cocaine framework is replaced by a phenylphosphonate moiety (**28**) that approximates the TS for ester hydrolysis.

Using a TS analogue strategy, a catalytic antibody (mAb15A10) was developed, which catalyzed the hydrolysis of cocaine to inactive ecgonine methyl ester. In a model of cocaine overdose, mAb15A10 protected rats from cocaine-induced seizures and sudden death in a cocaine dose-dependent fashion.[97] As further proof of the concept, the ecgonine methyl ester concentration was increased more than 10-fold in the plasma of rats treated with the monoclonal antibodies. In a model of cocaine self-administration, mAb15A10 completely blocked the reinforcing effects of cocaine in rats. These

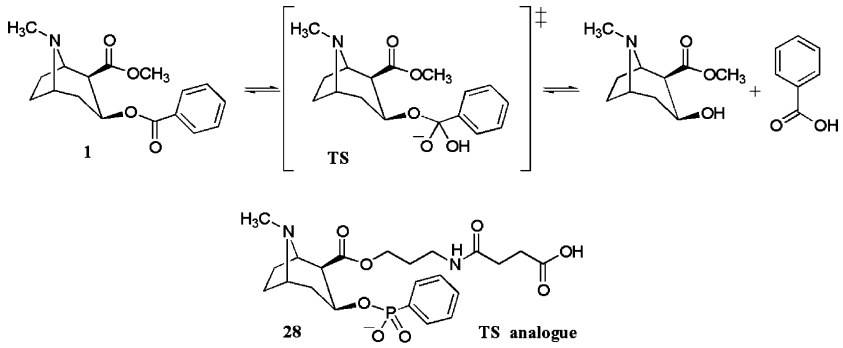

Figure 11. Hydrolysis of cocaine by catalytic antibodies; transition-state analogue **28**.

preclinical findings can be considered as proof of the general concept, but the approach needs further clinical development and evaluation.

Another protein-based approach to cocaine addiction treatment is via enhancing cocaine metabolism with naturally occurring enzymes such as human liver carboxylesterases hCE-1 and hCE-2, and butyrylcholinesterase (BChE).[98,8] BChE is a major cocaine-metabolizing enzyme present in the plasma of humans and other mammals.[8] Intravenous pretreatment of rats with 5000 IU of horse serum-derived BChE followed by administration of 17 mg/kg cocaine (intraperitoneally) produced a significant attenuation of cocaine-reduced locomotor activity. In a further proof of this approach, BChE altered the cocaine metabolic pattern to yield the nontoxic metabolite ecgonine methyl ester, instead of the usual benzoylecgonine metabolite. However, systemic administration of BChE compares unfavorably to other pharmacokinetic approaches to attenuating cocaine action including active immunization[4,91] and catalytic antibodies.[7] Although, unlike active immunization, which takes weeks to generate a useful antibody titer, BChE or cocaine antibody administration can produce protective effects within minutes after intravenous administration; active immunization however may be more effective at retarding cocaine from entering the CNS since these antibodies would be of higher affinity for cocaine.

An important caveat to this approach, however, is that the specificity rate constants (k_{cat}/K_m) of these cocaine-hydrolytic human esterases are several orders of magnitude below a diffusion-limited rate.[99,100] Not having evolved under selection pressure for cocaine hydrolysis, these enzymes accept cocaine serendipitously as part of a broad range of substrates. It therefore follows, that an enzyme, which has evolved to specifically hydrolyze cocaine would likely have a faster rate than esterases such as hCE-1, hCE-2, and BChE. A recently characterized bacterial cocaine esterase, CocE, was reported to hydrolyze cocaine faster than any other described cocaine esterase. Hydrolysis of the cocaine benzoyl ester was found to proceed with a $k_{cat} = 7.8\,s^{-1}$ and $K_m = 640\,nM$. Isolated from Rhodococcal strain (MB1) that grows in the rhizosphere soil of the cocaine producing plant *Erythroxylum coca*, CocE is one of only two known enzymes that are believed to have evolved under selection pressure for cocaine hydrolysis.[101] CocE catalyzes hydrolysis of the benzoyl ester of cocaine as the first step in a metabolic pathway that is capable of utilizing cocaine as the only source of carbon and nitrogen.[102] With a specificity rate constant that is orders of magnitude faster than any other known cocaine hydrolyzing enzyme or catalytic antibody, the absence of observable product inhibition and the ability to accept both cocaine and cocaethylene as substrates, CocE profiles as a promising addition to the generation of cocaine catalytic antibodies for addiction therapy. The success of its implementation in animal models of drug abuse and the hapten design to elicit cocaine catalytic antibodies remain to be examined.

8. Conclusion

Advances in our understanding of the underlying biology of cocaine addiction are affording new venues to procure the development of a still elusive effective therapy against this disease. Undoubtedly, the greatest challenge in this scientific endeavor is the inherent multifaceted nature of cocaine abuse with its complex psychosocial and neuropharmacological components. Immunotherapeutic strategies described herein offer a means with which to safely and effectively address relapse behavior, the most self-perpetuating component of cocaine dependence. Still with important challenges to overcome, such as titer surmountability and improved binding affinity, immunopharmacotherapy appears to be, to date, the most promising tool to address cocaine abuse, in concert with complementary therapeutic strategies that address the complex nature of this social affliction.

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